Quantifying acute physiological biomarkers of transcutaneous cervical vagal nerve stimulation in the context of psychological stress

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Abstract

Background: Stress is associated with activation of the sympathetic nervous system, and can lead to lasting alterations in autonomic function and in extreme cases symptoms of posttraumatic stress disorder (PTSD). Vagal nerve stimulation (VNS) is a potentially useful tool as a modulator of autonomic nervous system function, however currently available implantable devices are limited by cost and inconvenience.

Objective: The purpose of this study was to assess the effects of transcutaneous cervical VNS (tcVNS) on autonomic responses to stress.

Methods: Using a double-blind approach, we investigated the effects of active or sham tcVNS on peripheral cardiovascular and autonomic responses to stress using wearable sensing devices in 24 healthy human participants with a history of exposure to psychological trauma. Participants were

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exposed to acute stressors over a three-day period, including personalized scripts of traumatic events, public speech, and mental arithmetic tasks.

Results: tcVNS relative to sham applied immediately after traumatic stress resulted in a decrease in sympathetic function and modulated parasympathetic/sympathetic autonomic tone as measured by increased pre-ejection period (PEP) of the heart (a marker of cardiac sympathetic function) of 4.2 ms (95% CI 1.6–6.8 ms, p < 0.01), decreased peripheral sympathetic function as measured by increased photoplethysmogram (PPG) amplitude (decreased vasoconstriction) by 47.9% (1.4–94.5%, p < 0.05), a 9% decrease in respiratory rate (−14.3 to −3.7%, p < 0.01). Similar effects were seen when tcVNS was applied after other stressors and in the absence of a stressor.

Conclusion: Wearable sensing modalities are feasible to use in experiments in human participants, and tcVNS modulates cardiovascular and peripheral autonomic responses to stress.

Introduction

Traumatic stress is associated with activation of the sympathetic nervous system (SNS), and can be associated with lasting alterations in autonomic function and in extreme cases symptoms of posttraumatic stress disorder (PTSD) [1]. Re-exposure to traumatic memories can be associated with a re-activation of the SNS, which can lead to symptoms of PTSD [2–6]. Vagal nerve stimulation (VNS) effectively modulates autonomic nervous system function and thus represents a potential treatment option for PTSD [7,8], however, implementation is limited by the implantation procedure. Wide-spread implementation of VNS has been limited by invasiveness of the therapy and high costs typically not covered by medical insurance [9–11]. Transcutaneous vagal nerve stimulation (tVNS) devices applied to cervical or auricular portions of the vagus nerve potentially offer substantially enhanced feasibility and tolerability [12–16], but their effects on physiology are not well understood.

The vagus nerve is a complex neural structure that contains descending efferent fibers that regulate peripheral organs and autonomic nervous system activity, and ascending afferent fibers to the brain via the nucleus tractus solitarus (NTS) [7]. The NTS projects to other brain areas such as the amygdala, hippocampus, locus coeruleus, and prefrontal cortex that play important roles in emotion regulation and have been implicated in stress-related mental disorders, including PTSD [17,18]. Efferent fibers modulate cardiovascular function and peripheral autonomic tone, which can also be modulated by afferent fibers via brain areas with effects on these parameters including the prefrontal cortex and insula [19]. Electrical stimulation of the vagus nerve, using implantable devices (direct VNS), has been demonstrated to be efficacious for the treatment of epilepsy and refractory major depression, and is approved by the Food and Drug Administration (FDA) for the treatment of these disorders [10,20–26]. The effects of direct VNS on autonomic imbalance likely explains much of its efficacy for these disorders, as well as its applicability to cardiovascular

disorders [27,28]. The effects of direct VNS on enhancement of memory and neuroplasticity also suggest a role for treatment of cognitive disorders, stroke, and other conditions [29–34].

tVNS devices that target the auricular (taVNS) or cervical (tcVNS) portion of the vagus nerve have recently been developed that, due to their low cost and on-demand usability, have the potential to be widely implemented for rehabilitation, treatment of mental disorders, and performance improvement [35,36]. taVNS and tcVNS technologies are considered separately as they target different portions of the vagus nerve: the auricular branch is accessed from the ear and the cervical branch from the right or left side of the neck. Brain imaging studies reveal that taVNS devices can modulate the vagal afferents [15,37–39], along with studies that report improved vagal tone through heart rate, heart rate variability, and microneurography [40,41], increased salivary alpha amylase, and decreased salivary cortisol [42]. Beneficial outcomes have been noted by multiple groups on episodic migraine [39], epilepsy seizure frequency [14], major depression [43,44], and chronic tinnitus [45].

While fewer studies exist focused on tcVNS, several important results have been demonstrated. Imaging studies noted vagal afferents are accessible with tcVNS [46] and, recently, a multi-scale image-derived model of tcVNS was developed predicting the fiber activation due to tcVNS [47]. The downstream effects were observed in serum cytokines, chemokines, and cardiac vagal tone [48,49]. Clinically relevant outcomes for tcVNS were noted for trigeminal allodyna and migraine [50,51]. Analyses on the effects of taVNS and/or tcVNS on cardiovascular and autonomic function have produced mixed outcomes [40,48,51–53]. These studies used basic parameters such as heart rate (HR), heart rate variability (HRV) and blood pressure (BP), that are easy to attain, but are influenced by sympathetic and parasympathetic nervous systems along with subsequent peripheral vascular resistance, and therefore do not provide information on specific target pathways and physiological systems [54,55]. New advances in wearable sensing devices, incorporating seismocardiography, electrocardiography, ballistocardiography, movement, and peripheral vascular constriction, have improved specific assessment of sympathetic, parasympathetic, cardiovascular, and peripheral vascular function in conjunction with tasks such as mental stress, and could be applied to neuromodulation [56–58]. VNS results in changes in cardiovascular and peripheral function, reflected by a dynamic interplay between the activation of descending efferents and ascending afferents [59]. Due to this complex interplay, the effects of neuromodulation on *both* sympathetic and parasympathetic autonomic systems must be considered. Assessment of the effects of VNS on autonomic function also has clinical relevance as maladaptive autonomic regulation is the hallmark of many psychiatric disorders including PTSD.

Autonomic function plays a critical role in the stress response, but little is known about the effects of VNS on autonomic responses to stress. Exposure to traumatic events can produce strongly encoded intrusive memories associated with alterations in autonomic function that can persist in certain vulnerable individuals, and be associated with long-term changes in brain circuits involved in stress response, and possibly lead to PTSD [60,61]. Studying autonomic correlates of traumatic stress memories has clinical implications for patients with PTSD [62]. Both traumatic stress [63,64], and other stressors such as public speech or mental arithmetic [65–67] can be produced in the laboratory. These paradigms have been

shown to reliably produce behavioral and physiological responses consistent with a stress response [65–68], although clinically there are fundamental differences between traumatic stress recall and "neutral" mental stress paradigms. Due to the role of traumatic reminders (conditioned fear) in PTSD, traumatic stress paired with direct or transcutaneous VNS has been studied in animal models and humans. In animal models, direct VNS with cuff electrodes has been shown to lead to improvements in fear response and pathological neural activity [8,69,70]. Human subject studies reported improvements in vagal tone in patients with PTSD through taVNS [71]. Some groups, through subjective scales, have reported improvements in fear and worry responses for taVNS for healthy populations [52,72,73], but no improvements in high worriers [53]. No changes were observed in physiological indices based on HR and HRV for fear and worry studies for taVNS, and pairing the less commonly used tcVNS with stress has not been explored. In the current study, we examined the effects of tcVNS applied in tandem with acute traumatic and mental stress on autonomic function in real time as measured with cardiovascular, peripheral, autonomic, and respiratory changes following tcVNS or sham administration with or without acute stress. We previously presented preliminary data from the initial participants of the current sample of traumatized healthy human participants without PTSD showing that tcVNS modulates autonomic responses to stress as measured by systolic time intervals and blood volume pulse [74,75]. In this study, we extended our measurements to electrocardiography (ECG), seismocardiography (SCG), photoplethysmography (PPG), respiration (RSP), electrodermal activity (EDA), and blood pressure (BP) signals, comparing two groups of participants receiving either active or sham tcVNS stimuli in conjunction with exposure to the stress of personalized traumatic scripts, mental arithmetic, and public speech tasks.

Materials and methods

Human subjects study

The study was performed under a protocol approved by the institutional review boards of Emory University (#IRB00091171), Georgia Institute of Technology (#H17126), SPAWAR Systems Center Pacific, and the Department of Navy Human Research Protection Program. The study took place in Emory University School of Medicine between May 2017 and October 2018 (ClinicalTrials.gov # NCT02992899). Participants included healthy adults between ages 18–65 with a history of psychological trauma but without current posttraumatic stress disorder (PTSD) or other major psychiatric disorder. Participants were recruited and provided written, informed consent for participation. Fig. S1 presents the Consolidated Standards of Reporting Trials (CONSORT) diagram for the study, and Table S1 provides demographic data on the participants. Exclusion criteria were: pregnancy, traumatic brain injury (TBI), meningitis, active implanted device, current history of PTSD or other major psychiatric disorder including schizophrenia, schizoaffective disorder, bipolar disorder, severe major depression, bulimia or anorexia based on Diagnostic and Statistical Manual-5 (DSM-5) criteria [76] and the Structured Interview for DSM (SCID) [77], evidence or history of serious medical or neurological illness, post-menopausal status, positive toxicology screen, and carotid atherosclerosis. The Clinician Administered PTSD Scale (CAPS) was administered to evaluate for presence and severity of both possible current and lifetime PTSD [78] and participants who met criterion for current PTSD based

on the CAPS were excluded. Among 46 individuals who were screened for eligibility, six declined to participate and 13 did not meet inclusion criteria. The remaining 27 were randomized to active or sham stimulus. Data were not available in three participants due to technical problems or withdrawals. This study presents data obtained from 24 participants including 12 females. Mean age of the participants was 31 (±9 SD). Sample size was pre-determined with power analysis. The active group and sham group participants were similar in age and sex. The active group participants (n = 12) had a mean age of 29 (±7 SD) and included five females; sham group participants (n = 12) had a mean aged of 32 (±11 SD), with seven females. SCID was used to evaluate for possible psychiatric diagnosis other than the diagnoses in exclusion criteria. In this sample, four (17%) met criteria for past depression, one (4%) for past PTSD, two (8%) for generalized anxiety disorder, one (4%) for past panic disorder, two (8%) for past alcohol abuse or dependence, and one (4%) for a past history of history of drug abuse or dependence.

Study design

Each participant was asked to write their traumatic events; later, personalized voice recordings based on these scripts were prepared using methods previously described [79]. The protocol consisted of three subsequent days for each participant, Fig. 1 presents the details for each day. The first day included six traumatic recall scripts and six neutral scripts presented audibly through headphones to participants inside a high-resolution positron emission tomography (HR-PET) scanner at 20 °C temperature (Fig. 1A), starting approximately at 8AM. All scripts were 60 s in duration. The neutral scripts were designed to induce positive feelings to the participant, such as the description of pleasant scenery, designed for the imaging part of the study. Traumatic scripts included personalized traumatic memories. Stimulation (active or sham) was applied immediately after the termination of the personalized traumatic script for 2 min by a research associate. On the same day two stimulation administrations (active or sham) were applied without any stressor. The second and third days were identical to each other, at 25 °C temperature (Fig. 1B), starting approximately at 8:30AM: First, the participants underwent a public speech task for which they were required to provide a 2-min long defense statement in a scenario where they were accused of theft. After hearing the scenario details, they were given 2 min to prepare their defense and 2 min to present their statement. Stimulation was applied immediately after the public speech task. Later, the participants rested for 8 min in silence. At the end of the 8 min, the participants were given another task for which they were required to answer series of arithmetic questions for 3 min. A researcher provided negative feedback for incorrect answers and delayed response times. A second stimulation was applied immediately after the arithmetic task. After two mental stressors and two stimulation administrations, the participants were given a 90-min break. After the break, another stimulation was administered without any stressor.

Blinding

The participants were randomized into either active tcVNS or sham stimulus groups with an online randomizer using simple randomization. The devices were pre-numbered by the manufacturer who were not involved in the research, and random allocation was conducted by an individual who did not take part in enrollment, data collection or analysis. Enrollment

was done by clinical staff. The participants and clinical staff were blinded to the stimulus type, and each of the participants received only one type of stimulus. The researchers, who were also blinded to the stimulus type, conducted the questionnaire assessments, data collection, signal processing, and parameter extraction. Statistical analyses were carried out by a biostatistician who did not take part in data collection or processing. Stimulus grouping (active or sham) was un-blinded for the interpretation of statistical analysis.

Transcutaneous cervical vagal nerve stimulation

Both active tcVNS and sham stimuli were administered using hand-held devices (GammaCore, ElectroCore, Basking Ridge, New Jersey) with identical placement and operation. tcVNS or sham was applied using collar electrodes on the left side of the neck. The treatment area on the neck was located by finding the pulse on the carotid artery for each participant (Fig. 2B). Conductive electrode gel (GammaCore, ElectroCore, Basking Ridge, New Jersey) was used to maintain good contact between the skin and the electrodes. Active tcVNS devices produce an AC voltage signal consisting of five 5 kHz sine pulses, repeating at a rate of 25 Hz. Sham devices produce an AC biphasic voltage signal consisting of 0.2 Hz square pulses that delivers a mild buzzing sensation similar to the active device but does not result in stimulation of the vagus nerve. Both active and sham device operation stops automatically after 120 s. The stimulation intensity was adjustable using a roll switch that ranged from 0 to 5a.u. (arbitrary units) with a corresponding peak output ranging from 0 to 30 V (~0–60 mA) for active tcVNS, and from 0 to 14 V (~0–60 mA) for sham device. During each application, the stimulation intensity was increased to the maximum the participant could tolerate, without pain. At the start of stimulation, the intensity was increased gradually until each participant instructed to stop. The stimulation continued at the selected intensity. The amplitude levels participants received were 18 V (±4.8 SD) for active tcVNS, and 12.6 V (±2.8 SD) for sham stimulus.

Physiological monitoring

Physiological data were collected by the measurement of the following signals: 3-lead electrocardiography (ECG), respiration (RSP), seismocardiography (SCG), photoplethysmography (PPG), electrodermal activity (EDA), and blood pressure (BP). Fig. 2A shows the test setup employed for each participant. The ECG, RSP, PPG, and EDA signals were measured using wireless Bionomadix RSPEC-R and PPGED-R amplifiers (Biopac Systems, Goleta, CA). Adhesive Ag/AgCl electrodes were used for ECG recording. A respiration belt was used to measure thoracic expansion and contraction while breathing in order to measure RSP signal. For SCG measurement, a low-noise 356A32 accelerometer was used on the mid sternum (PCB Electronics, Depew, NY). Only the SCG signals in the dorsoventral direction were used in this study. Transmissive PPG measurement was taken from the index finger. EDA measurement was taken from the same hand where the PPG measurement was taken, using the inner palm. An isotonic electrode gel (GEL101, Biopac Systems, Goleta, CA) and pre-gelled isotonic electrodes (EL507, Biopac Systems, Goleta, CA) were used for EDA recording. All data were transmitted to the Biopac MP150 16-bit data acquisition system at a sampling rate of 2 kHz. Non-continuous systolic (SBP) and diastolic blood pressure (DBP) values were recorded periodically with an Omron blood pressure cuff during the rest, all stressors, and stimulation administrations.

Signal processing & parameter extraction

The signal processing and parameter extraction were carried out in MATLAB (R2017b, Natick, MA). The following parameters were extracted: heart rate (HR), pre-ejection period (PEP), amplitude of PPG, pulse arrival time (PAT_{FOOT}, PAT_{PEAK}), respiration rate (RR), width (RW), respiration prominence (RP), low frequency and high frequency heart rate variability (LF HRV, HF HRV), skin conductance level [80], skin conductance response [81], frequency of non-specific skin conductance responses (f_{NSSCR}), and latency of skin conductance response (L_{SCR}).

Pre-Processing: Fig. 2C shows sections from collected physiological signals from a participant and the parameters computed from these signals. The ECG, SCG and PPG signals were filtered with finite impulse response [77] band-pass filters, with cut-off frequencies 0.6–40 Hz for ECG, 0.6–25 Hz for SCG, and 0.4–8 Hz for PPG, respectively, to preserve the waveform shape and cancel the noise outside their bandwidths [82,83]. The phasic component of EDA (for computing the parameters related to skin conductance response) was obtained using an FIR 0.15 Hz equiripple high-pass filter. The slowly varying RSP signal was used as is, as the module applies 10 Hz low-pass filter internally. The R-peaks of the ECG signals were detected using thresholding, and were used to calculate HR, HRV. SCG and PPG signals were ensemble averaged according to the R-peaks, using beat lengths of 150 ms for SCG and 600 ms for PPG. These lengths were sufficient to detect the fiducial points of each SCG and PPG beats. To reduce the effects of motion artifacts on the individually segmented beats, exponentially weighted moving ensemble averaging of successive beats was implemented for some parameters described below [82]. Exponentially decreasing weighting gives more emphasis to the more recent beats, while still providing noise reduction based on the averaging. Additional beat exclusion criteria were checked inside the algorithms, such as the identification of unrealistic timing intervals or unexpected morphology that may be caused by motion artifacts or momentary noise on the signal. For each parameter extraction, the beats were monitored to ensure that the time points were located correctly and the beats had acceptable morphology. The reader is referred to Fig. S2 for sample step-by-step PPG amplitude extraction from ECG and PPG signals. Similar ensemble averaging and feature extraction to extract amplitude and timing intervals were used for all continuous beat-by-beat signals.

Pre-Ejection Period: PEP, measured by the latency between the start of electrical depolarization of the ventricles to the opening of the aortic valve, is a non-invasive measure of cardiac contractility and cardiac sympathetic activity [84]. PEP provides limited insight on its own regarding baseline sympathetic tone or contractile state of the heart – two people with the same level of contractility may have different baseline PEP values due to differences in preload and afterload, which also impact the time it takes for the heart to proceed through isovolumetric contraction to systolic ejection of blood. However, *changes* in PEP have been associated with *changes* in contractility, specifically with a *decrease* in PEP indicating an *increase* in contractility. The increase in contractility leads to an increase in the maximal derivative of left ventricular pressure during isovolumetric contraction (i.e., dP/dt_{max}), and thus leads to a shortened PEP. In this work, we are examining the acute changes in PEP either associated with a stressor (e.g., traumatic stress) or tcVNS, or both, and thus

are using PEP as an indicator of acute changes in cardiac contractility and thus sympathetic tone. The SCG signal provides high quality PEP estimation when combined with the ECG, computed by the time difference between the R-peak of the ECG to the second peak in SCG beat (aortic opening, AO point), known as R-Ao [57]. R-Ao values were computed following a three-beat exponential moving averaging procedure for noise reduction.

PPG Amplitude and Pulse Arrival Times: The PPG signal is known to be affected by sympathetic and vasomotor activity [83], therefore different parameters were extracted using this signal. Firstly, as a measure of peripheral sympathetic activity and vasomotor activity at the area of signal collection (index finger), the amplitude of each PPG beat was extracted. Secondly, pulse arrival time (PAT), representing the time delay from the electrical depolarization of the heart to the arrival of the pressure wave to the index finger was calculated from two reference points [85]. The first reference point was the foot of PPG signal, which was located by finding the maximum of the second derivative of the pulse wave before the maxima (PAT_{FOOT}). The second reference point was the peak (maxima) of the PPG signal (PAT_{PEAK}). A time constant of five beats was used for both PAT_{FOOT} and PAT_{PEAK} calculation.

Respiratory Measures: The respiratory parameters extracted were respiratory rate (RR), respiration width (RW), and respiration prominence (RP). Due to the loosening of the respiration belt over time while the participant was inside the PET scanner, the respiration signal occasionally had a DC offset. To remove this offset, a sixth order polynomial was fit to the signal in each interval (i.e. rest or stress), and the signal was detrended. From the detrended signal, the peaks representing inhalation and exhalation were located using thresholding. The rate of the peak appearance was extracted as RR. RR was considered as a continuous index of parasympathetic activity [86]. For RW, the width of each peak was computed as the distance between the points to the left and right of the peak, where the descending signal intercepts a horizontal reference line. The reference line was positioned beneath the peak at a vertical distance equal to half the peak prominence. The points themselves were found by linear interpolation. RP measured the prominence of a peak, i.e. how much the peak stands out due to its intrinsic height and its location relative to other peaks. It was calculated as the minimum vertical distance that the signal descends on either side of the peak before either climbing back to a level higher than the peak or reaching an endpoint.

Heart Rate Variability Measures: Two techniques were used to extract multiple HRV measures: Frequency-domain analysis and joint time-frequency analysis (Poincaré method). The first method, frequency-domain HRV, is the most commonly studied method for quantifying the sympathetic and parasympathetic branches of the autonomic nervous system, obtained from the non-constant R-R intervals from ECG R-peaks [87]. While the power in the high-frequency range (HF HRV, 0.15–0.4 Hz) is considered a measure of parasympathetic activity for humans, the low-frequency portion (LF HRV, 0.04–0.15 Hz) is mostly used for assessing the changes related to both sympathetic and parasympathetic influences [87]. The ratio of the two power bands (LF/HF) is often considered as a measure of sympathetic tone, while there are discrepancies in the literature [86,88]. For the second

HRV analysis (Poincaré method), three standard indices were computed from the scatter plot of each R-R interval ($R\text{-}R_n$) versus the next R-R interval ($R\text{-}R_{n+1}$). In this procedure, an ellipse is fitted to the line-of-identity of the scatter plot ($R\text{-}R_n$ versus $R\text{-}R_{n+1}$). Three indices were extracted from the fitted ellipse: standard deviation of points perpendicular to the axis of line-of-identity (SD1), standard deviation of points along the axis of line-of-identity (SD2), and their ratio (SD1/SD2). SD1 measures short-term HRV which correlates with baroreflex sensitivity (BRS, change in the inter-beat interval duration per unit change in BP) and HF HRV. SD2 measures short- and long-term HRV and correlates with BRS and LF HRV. The ratio SD1/SD2 (the unpredictability of R-R intervals) is an indicator of the autonomic balance [89,90]. For both frequency-domain HRV and Poincaré analyses, ECG signals from the start and end of the days (longer than 5 min), ECG signals during stress (one to 3 min), stimulation (2 min), and post-stimulation (two to 8 min) were used. For each interval, the ECG signal was inspected visually to avoid ectopic, noisy beats and arrhythmias.

Despite the wide use of these HRV indices, there is still ambiguity in the research community emerging from the lack of clear documentation, validation, and standardization of different HRV signal processing methods. Here for HRV analysis, we used a MATLAB-based open source HRV toolbox that was previously validated with a variety of HRV measurement techniques and platforms to calculate LF HRV, HF HRV, LF/HF HRV, SD1, SD2, SD1/SD2 [91].

Electrodermal Activity Measures: The EDA signal is composed of two main components. The slow tonic component (skin conductance level, SCL) shows the general trend of the signal. The faster tonic component (skin conductance response, SCR) is superimposed onto the tonic component. Electrodermal activity parameters extracted were SCL, SCL slope, SCR, frequency of non-specific skin conductance responses (f_{NSSCR}), and latency of skin conductance response (L_{SCR}) [92]. For SCL, the DC level of EDA signal was extracted and the mean, minimum, maximum, standard deviation, slope of the first order polynomial fit (SCL slope), and area under curve properties were derived. SCR was analyzed in a similar manner to SCL. The peaks in SCR were located by thresholding, and the number of peaks per interval was computed to calculate f_{NSSCR}, excluding the first peak in the signal which corresponds to a specific event (i.e. stress start instance). For L_{SCR}, the latency from the start of the interval to the first peak appearance was calculated. The determination of the minimum peak amplitude was required to define the response occurrence. Although a minimum of 0.05 μS is common with hand scoring of SCR responses, this threshold is largely task- and subject-specific, and can be as low as 0.01 μS [92]. We determined the minimum peak amplitude to be two times the rest SCR mean amplitude for each participant, resulting in a mean of 0.06 ± 0.03μs for this study.

Statistical analysis

We compared participant characteristics between active and sham group using student t-tests (for normal continuous variables), Wilcoxon rank-sum tests (for non-normal continuous variables), and chi-squared tests (for categorical variables), as shown in Table S2. To understand the relative changes in the physiological parameters, data were separated into

intervals reflecting the baseline of the corresponding day, stress, stimulation (active or sham), and post stimulation. Absolute and percent changes from the baseline state for each interval were computed and compared between-group differences across the intervals. For physiological parameter intervals (except HRV), data from 1 min of baseline rest, first 30 s of stress, last minute of stimulation, and 1 min from post-stimulation (3 min after the stimulation stops) were used. For speech and mental arithmetic tasks which corrupt the respiration waveform due to vocalization, the respiration beats just before the subjects start speaking were extracted as respiratory data during these stressors. These intervals correspond to the end of speech preparation (just before the subject starts speaking after 2-min preparation), and the interval just after the subjects heard the first mental arithmetic question (before answering). For non-continuous BP analyses, similarly SBP, DBP, PP values measured during baseline, stress, stimulation, and post-stimulation were used. Longer intervals for HRV measures were used to comply with the standards. The extracted parameters were evaluated with respect to the corresponding baseline values for each day, either as a ratio with baseline (percent changes) or subtraction from the baseline (absolute changes), for each interval. HRV indices were also evaluated as raw values for each interval. Data in bar plots were represented as mean ± 95% confidence interval, CI plotted from the raw unadjusted values. To evaluate if device type (active vs. sham) was associated with changes in parameters from the baseline value, we used mixed models with repeated measures that included random effect for each participant using unstructured correlation matrix (i.e., multiple traumatic scripts from the first day, two stimulations without acute stress on the first day, two stimulations without acute stress after a 90-min break on the second and third days, two stimulations followed by two public speech or two mental arithmetic tasks), and adjusted for age in the models. In a sensitivity analysis, we also tested the significance of the interaction between device type and time variable. Statistical analyses on both percent and absolute changes were carried out in all the models. The beta coefficients (β) from the mixed models indicate the adjusted average percent or absolute differences in the changes of parameters from the corresponding rest values, comparing active vs. sham device types. β were reported along with 95% CI and P-values in results and figure captions. A two-sided $p < 0.05$ denoted statistical significance. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and MATLAB (R2017b, Natick, MA).

Results

tcVNS has a similar effect on SNS activity both in the presence and absence of stress

To understand the physiological changes induced only by active or sham stimulation, the protocol included two stimulation administrations in the absence of traumatic scripts or mental stress (mental arithmetic and public speech) tasks, after a 90-min break from the mental stress protocol on the second and third days (Fig. 3 shows the data from the unadjusted raw changes from the baseline state during stimulation and post-stimulation intervals, results were expressed as mean values, 95% confidence intervals, p-values obtained after adjustments). Stimulation without stress tasks resulted in differences in physiological biomarkers associated with sympathetic tone: PPG amplitude (Fig. 3A, measurement of peripheral vasoconstriction, inversely related to peripheral sympathetic

activity) increased (indicating relative vasodilation and decreased sympathetic activity) during stimulation by 78.6% (95% CI, 0.5–156.7%, p = 0.049), and following stimulation by 95% (15.7–174.2%, p = 0.021) after adjustments in the active tcVNS group relative to the sham group. The pre-ejection period (PEP, Fig. 3B, inversely related to cardiac sympathetic activity) increased following stimulation by 3.3 ms (0.2–6.3 ms, p = 0.035) after adjustments in the active group compared to the sham group, indicating a decrease in cardiac contractility and sympathetic activity. Electrodermal activity slope (SCL slope, Fig. 3C, related to sympathetic activity) decreased during post-stimulation by −0.013 μS/s (−0.024 to −0.003 μS/s, p = 0.014) after adjustments in the active tcVNS group relative to the sham group.

tcVNS modulates autonomic tone following exposure to personalized traumatic scripts

Stimulation following exposure to personalized traumatic scripts revealed marked changes in autonomic reactivity between the active and sham groups. Fig. 4A–C illustrates changes in physiological parameters from the baseline state for the three intervals: traumatic stress, stimulation, and post-stimulation, data shown from unadjusted raw values. There were no significant differences in peripheral vasoconstriction measured by PPG amplitude during traumatic scripts between groups. There was an increase in PPG amplitude (indicating relative vasodilation and decreased peripheral sympathetic activity) during stimulation delivered immediately at the termination of traumatic scripts which persisted after the end of stimulation in the active versus the sham group. PPG amplitude was 43.7% higher (3.1%–84.3%, p = 0.036, Fig. 4A) during active versus sham stimulation and 47.9% higher (1.4%–94.5%, p = 0.044) in the post-stimulation interval after adjustments. As for PEP, there were no significant differences in PEP during traumatic scripts and during stimulation between groups. In the post-stimulation interval, an increase in PEP (indicating decreased cardiac sympathetic activity) was observed in the active versus sham group with an adjusted difference of 4.2 ms (1.6–6.8 ms, p = 0.003, Fig. 4B). Respiratory rate (RR) was similar between tcVNS and sham groups during traumatic scripts and stimulation, with an adjusted decrease in the active group relative to sham of −9% (−14.3% to −3.7%, p = 0.002, Fig. 4C) during post-stimulation indicating a release of parasympathetic activity.

Effects of tcVNS on PPG amplitude and respiration rate following mental stress

There were no statistically significant differences during the public speech task between the active and sham groups in PPG amplitude, RR, respiration prominence (RP), SCL slope (Fig. 5A–C, F). PPG amplitude increased during post-stimulation in the active group compared to sham by 61.3% (17.3%–105.3%, p = 0.009, Fig. 5A) after adjustments. RR decreased in the post-stimulation in active versus sham by an adjusted difference of −11.3% (−20.3% to −2.3%, p = 0.017, Fig. 5B). RP decreased during stimulation in active versus sham by −25.4% (−47.9% to −3%, p = 0.028, Fig. 5C) after adjustments. Lastly, SCL slope decreased during stimulation in active versus sham by −0.014 μS/s (−0.026 to −0.001 μS/s, p = 0.027, Fig. 5F) after adjustments.

Similar to public speech, there were no difference between active and sham groups during the mental arithmetic stress task in PPG amplitude or RR. Active stimulation relative to sham resulted in an adjusted increase in PPG amplitude of 95.8% (32.3%–159.2%, p =

0.005), with a post-stimulation adjusted increase of 70.4% (30.8%–110%, p = 0.001) (Fig. 5D). Following active tcVNS there was a decrease in RR of −14.6% (−24.8% to −4.3%, p = 0.007, Fig. 5E) after adjustments. Increased PPG following mental stress tasks and tcVNS indicates decreased peripheral sympathetic activity while decreased RR suggests a decrease in parasympathetic withdrawal. As for the two administrations without acute stress on the first day, PEP in active group compared to sham increased by 7.2 ms (p = 0.027) after adjustments following stimulation. There were no other marked differences in heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), respiration width (RW), low-frequency heart rate variability (LF HRV), or high-frequency HRV, low-to-high HRV ratio (LF/HF), SD1, SD2, SD1/SD2, pulse arrival time (PAT), other parameters related to electrodermal activity, such as skin conductance level (SCL), frequency of non-specific skin conductance responses (f_{NSSCR}), and latency of skin conductance response (L_{SCR}) that could distinguish active tcVNS and sham stimulation.

Discussion

This study demonstrated the feasibility and utility of quantification of cardiovascular and peripheral autonomic nervous system function using wearable sensing devices in conjunction with administration of tcVNS and a sham control and stressful tasks. tcVNS minimized sympathetic activation and/or withdrawal of parasympathetic tone following exposure to stress based on a range of physiological parameters. This was observed for different kinds of stressors, including exposure to recordings of personalized traumatic memory scripts, and "neutral" or "mental stress" tasks including mental arithmetic and public speech stress tasks. The findings suggest that wearable sensing devices could be used as real-time non-invasive physiological biomarkers of tcVNS to predict treatment efficacy and/or provide empirical evidence of proper tcVNS administration.

tcVNS shows effects in multiple physiological biomarkers

Active tcVNS compared to the sham group resulted in a decrease in peripheral and cardiac sympathetic activation for tcVNS alone as measured by increased PPG amplitude, increased PEP, and decreased SCL slope. There was also a reduction in peripheral sympathetic activation with tcVNS applied after both traumatic script and mental stress tasks as measured by increased PPG amplitude, and decreased cardiac sympathetic activation after traumatic scripts (but not mental stress) based on increased PEP. In a complementary manner, tcVNS resulted in reduced parasympathetic withdrawal after both traumatic scripts and mental stress tasks based on reduced RR. tcVNS also decreased SCL slope when followed by public speech task (but not arithmetic or traumatic stress). The use of various stressors revealed task-specific changes in autonomic nervous system activity: while an increase in PEP was observed for tcVNS when applied following a traumatic stressor, an increase in PEP was not observed upon stimulation for mental stressors. Similarly, reduction in SCL slope was observed for stimulation without acute stress and stimulation following public speech only. On the other hand, tcVNS resulted in increases in PPG amplitude and decreases in RR when applied after both traumatic script and mental stress tasks. Thus, there is not a single biomarker of tcVNS, rather its efficacy could be revealed from signals that are related to different pathways of autonomic reactivity to different types of stressors.

Changes in PPG-amplitude versus lack of changes in blood pressure

Increased PPG amplitude with tcVNS was one of the most consistent results across the various stressful tasks in this study. However, a myriad of factors is involved in affecting the amplitude of PPG signals, and thus associating changes in PPG amplitude changes with a particular underlying physiological origin is not straightforward. To the first order, the two main factors influencing PPG amplitude are pulse pressure and arterial compliance [93]. Thus, it is important to note that in this study we did not observe differences between the active tcVNS and sham groups in systolic, diastolic blood pressure, and pulse pressure for any of the intervals – this indicates that the changes in PPG amplitude that were significant, and quite substantial, were linked to local changes in arterial tone associated with vasoconstriction and vasodilation. Accordingly, the effects of tcVNS on PPG amplitude may be attributed to sympathetic regulation of vascular tone.

No effects of tcVNS on heart rate (HR) or heart rate variability (HRV)

The current study did not find differences between the active tcVNS and sham groups in ECG-based measurements of heart rate (HR) or heart rate variability (HRV) either for short intervals during and after stimulation, or for whole day measurements. HRV is commonly used as a proxy measure for peripheral autonomic function [87]. HRV measures have limitations, however, in terms of specificity and validity of assessment of specific aspects of sympathetic and parasympathetic autonomic activity [94]. Frequency-domain based HRV measures are convenient to measure, provided that the recording is long enough (at least 3–5 min) without ectopic and noisy beats. However, there has long been a debate about the relative contribution of sympathetic and parasympathetic activity to LF HRV, and there is general agreement that it is not a specific measure of sympathetic activity alone [86,95,96]. HR is also not specific, since changes in HR might reflect either an increase in SNS or decrease in PNS. HR carries information solely on the electrical activity, while PEP (controlled by the contractile force in the heart) incorporates information on electromechanical coupling of the heart [97–99]. Our findings suggest that tcVNS has specific effects on sympathetic and parasympathetic function distinct from other cardiovascular parameters as shown by specific effects on PEP, RR, PPG amplitude, and SCL slope, but not on HR, HRV, or EDA measures other than the slope. The HR measurements are not significantly different between active and sham groups for any of the intervals analyzed (Fig. S3). The changes in PEP thus provide more information than changes in HR alone. Our findings are also consistent with studies observing no HRV or EDA changes (SCR) following auricular VNS in combination with subjectively measured fear and anxiety [52,72,73]. A recent study on the effects of tcVNS with a noxious stressor (thermal stimuli) reported difference in EDA-related changes in active and sham group, specifically in SCL slope and latency of SCR (L_{SCR}) [100]. Our analysis has shown difference for SCL slope, but not for latency of SCR.

Use of seismocardiography (SCG) in the assessment of effects of tcVNS on peripheral autonomic function

The current study found PEP, a measure of sympathetic activity, to be useful in the assessment of the effects of tcVNS on autonomic function. PEP has been studied as a

measure of cardiac sympathetic activity (or cardiac contractility), along with comparisons with HRV, EDA, and plasma catecholamines [84,97–99,101–103]. However, PEP is used less commonly in practice in clinical studies due to the need for multiple electrodes and the addition of another sensing modality (impedance cardiography or ICG) along with ECG. We observed in our study that tcVNS administration creates electrical stimulation artifacts on ICG signal as the stimulation bandwidth and ICG signal bandwidth coincide with each other (Fig. S4, SCG versus ICG during tcVNS), hiding the fiducial point to extract the PEP (known as B-point, representing the opening of aortic valve) [84]. SCG is a viable option to calculate PEP in clinical studies that use tcVNS as it is a mechanical signal reflecting the chest-wall vibrations of the heart, hence the electrical stimulation does not affect the waveform shape. Beat-by-beat analysis during the treatment is possible with SCG-derived PEP. SCG also does not require electrodes, unlike ICG. tcVNS in the current study induced robust changes in PEP with or without stress, and across of a broad range of different stressful tasks.

Translation to populations afflicted with maladaptive autonomic regulation

Patients with PTSD suffer from recurrent and intrusive thoughts about traumatic events. Our results regarding the use of tcVNS in tandem with traumatic stress motivate possible translation to PTSD populations, in the clinic or at-home, as an acute treatment for these recurrent memories [60]. However, these results, which focus on the physiological biomarkers of tcVNS for individuals with prior psychological trauma, do not yet address the question of how the population with maladaptive autonomic regulation (i.e. patients with PTSD) would respond to tcVNS treatment. Nevertheless, it is known that individuals with PTSD have abnormal oscillations in autonomic state and show hyperarousal after recalling traumatic memories supporting either elevated sympathetic activity or withdrawal, as observed by physiological signals [104], brain imaging [62], or serum biomarkers [105]. PTSD patients suffer from exaggerated responsivity to reminders of traumatic memories, and the changes induced by tcVNS observed in traumatized persons without PTSD may potentially be observed in this population as well. Our findings on tcVNS hold promise for PTSD as there is preclinical evidence for direct VNS to enhance he extinction of conditioned fear [8,69,70], and clinical evidence for taVNS to improve vagal tone in patients with PTSD [71], though with different stimulation targets, direct VNS and auricular VNS, respectively.

Limitations

The following limitations should be noted for this study. Prior animal studies initiated direct VNS or sham before the initiation of the fear-related stimulus [8,32]. Other studies in human subjects initiated taVNS or sham before or during the stimuli [52,71,73]. Therefore, stimulation prior to stress and concurrently with stress appear to improve the pathological response based on previous studies. This study employs a reactive acute treatment approach as stimulation administrations were applied right after the stressors ended. Subjects were instructed, however, to form an image from the traumatic scripts in their mind and hold it, and stimulation was applied immediately at the end of the script. Our prior experience with traumatized subjects including those with PTSD demonstrated that upsettedness typically continues after the termination of the script, stress- or fear-related task [63,106]. Therefore, we believe that the stimulation was applied at the peak of the behavioral effects of the task.

Future studies should investigate the effects of preemptive versus reactive stimulation in the context of traumatic stress.

Due to the clinical nature of this study, the target engagement of the cervical vagus nerve could not be validated directly. This study relies on previous literature that reported the ability to reach the vagal afferents using tcVNS [46,47]. We replicated the stimulation application reported in Ref. [46] throughout the protocol, by locating the carotid artery as an anatomical reference. Although variation exists regarding the location and topographical anatomy of the cervical vagus nerve, a recent cadaveric study reports that cervical vagus nerve can be visualized in a 35×35 mm distance lateral of the laryngeal eminence and posterior to the skin of the neck, which typically falls under the area the electrodes are placed [107].

A natural restriction of this study is the possibility of therapeutic effects from traumatic exposure (traumatic stress rehearsal) [108]. Fig. 4 combines data from all six traumatic stress scripts per subject, showing increases only in RR during traumatic stress. To show how the subjects respond to traumatic stress initially, we also analyzed only the first traumatic script responses for the primary outcome variables, excluding all other repetitions, for each subject (see Fig. S5). It is seen that HR and RR increase, PPG amplitude decreases during traumatic stress. The lower stress reactivity in Fig. 4 might be due to the therapeutic effects of the repetitions as the data were merged from six traumatic scripts per subject (repetition numbers were included in our statistical analyses). Nevertheless, as our study focuses on tcVNS effects on stress, our main consideration was whether the active and sham groups received comparable amounts of stress. We did not observe significant differences in stress responses, which was an essential requirement to evaluate the effects of tcVNS on the recovery from stress. Therefore, although repeated exposure might change stress reactivity over the time, the reactivity remained similar between the active and sham groups, which facilitated comparison of the effects of active and sham stimulation.

The functional relevance of the PPG amplitude results could be attributed to changes in total peripheral resistance (TPR) or pulse pressure (PP), however there is no direct linear correlation to either. The PPG signal is an optical measurement, the amplitude of which is determined by the Modified Beer-Lambert Law [93]. PPG amplitude reflects the expansion and contraction of the vessel diameters in the region (index finger) being illuminated by the light source. This expansion and contraction of vessel diameter is proportional to both PP and arterial compliance. Compliance is the change in a vessel's volume for a given change in PP. Thus, while directional relationships between PPG amplitude changes and TPR can be quite informative, the attribution of a given change in amplitude to a particular change in TPR is complex. Nevertheless, the study did not find remarkable differences in non-continuous BP measures (SBP, DBP, PP) or pulse arrival time (PAT). This is an interesting result considering the relationship of PPG and BP waveforms [56,83]. PPG measurements (hence the extracted PPG amplitude and PAT) were continuous, and thus beat-by-beat assessment was feasible —a desirable measurement for the acute characteristics of this study. BP measurements were taken through a blood pressure cuff, and hence BP changes at beat-by-beat level could not be assessed. Future studies should examine whether continuous BP is affected by tcVNS.

While assessing the mental stress reactivity to tcVNS, it is important to clarify that the active and sham groups reacted similarly to mental stressors, which permits the comparison of stress response upon stimulation between the groups. The public speech task is a version of Trier Social Stress Test [109]. Traumatic stress protocol, public speech, and mental arithmetic tasks have been verified multiple times to induce significant psychobiological and cardiovascular responses on human subjects [62,110–112]. In this study, similar responsivity between the groups were seen in the measures analyzed during the stressors. The groups showed no significant difference during stress intervals in any of the cardiovascular, peripheral, electrodermal activity measures.

Conclusion

In summary, our investigation demonstrates that tcVNS has effects on peripheral autonomic function that can be feasibly and reliably measured with wearable sensing devices. Specifically, tcVNS both in isolation and following exposure to stress reduces sympathetic and enhances parasympathetic function, leading to a modulation in autonomic tone. These physiological biomarkers may be useful for long-term monitoring of tcVNS in the home setting to assess adherence and accuracy of neuromodulation treatments and to provide subject-specific dosage recommendations for tcVNS therapy. tcVNS also minimizes sympathetic activation in response to stress, which suggests that it may have clinical applications to stress-related psychiatric disorders characterized by increased sympathetic activity that is correlated with symptoms of these disorders [113–118]. The fact that tcVNS reduces or blocks sympathetic arousal associated with exposure to personalized traumatic scripts suggests a clinical application to patients with PTSD in the context of modulation of indelible traumatic memories and possible enhancement of neuroplasticity and/or facilitating extinction of conditioned responses to reminders, which were previously studied in preclinical literature through direct VNS with implantable devices [8,32,69,70,119,120]. Although not assessed in the current study, emerging findings of the beneficial effects of direct VNS on cognition and memory suggest other possible benefits of tcVNS for patients with stress-related psychiatric disorders [121]. tcVNS could have a potentially broad impact in the domains of human performance and mood improvement, and wearable sensing devices can be used to quantify the stimulation. This could be applicable to other clinical and neuroscience research environments and in general wearable bioelectronic medicine, for patients with or without psychiatric disorders or other medical conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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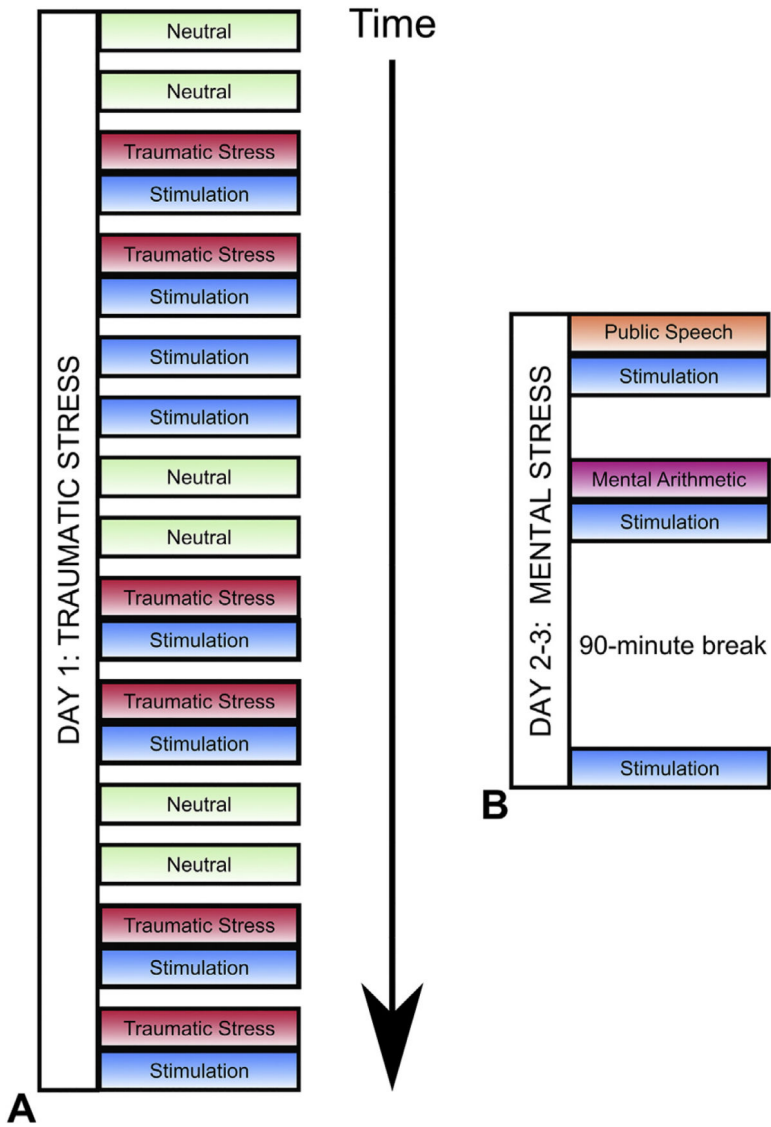
Fig. 1.
Protocol description. **(A)** The first day included traumatic stress through headphones. After each traumatic stress prompt, stimulation (active or sham) was applied immediately. **(B)** Second and third days included two types of mental stress, public speech and mental arithmetic. After each stressor, stimulation was applied immediately. After a 90-min break from the mental stress protocol, participants received tcVNS or sham without acute stress.
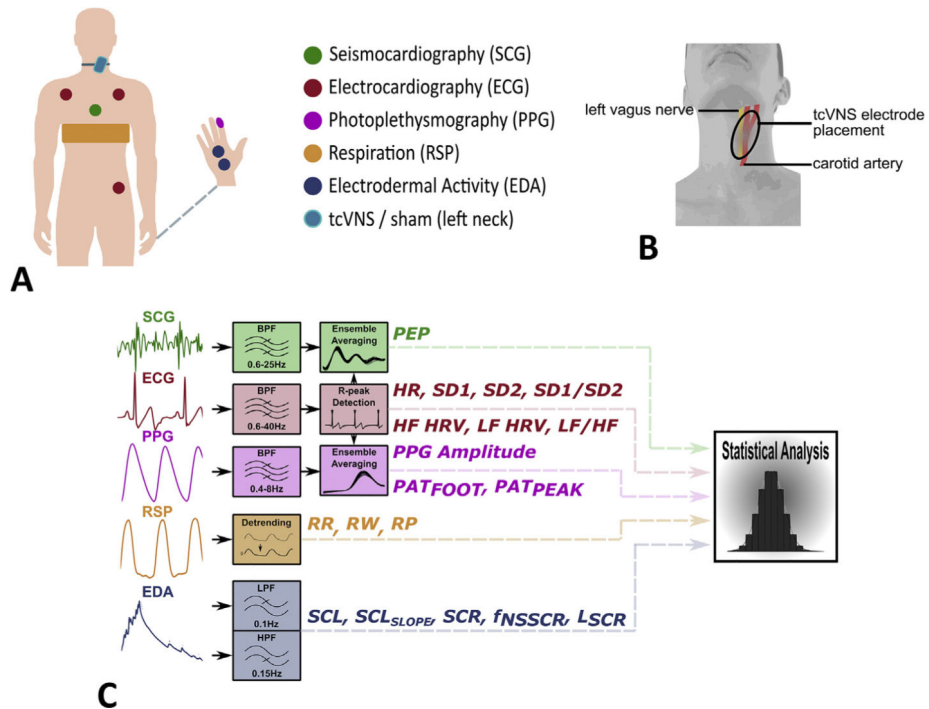
Fig. 2.
Data collection and signal processing summary. **(A)** Non-invasive sensing modalities shown on participant, active or sham stimulation was applied from left neck. **(B)** Representation of relative locations of left carotid arteries and left vagus nerve. tcVNS electrodes were placed onto the area where the carotid pulsation was located. **(C)** Signal processing and feature extraction summary.
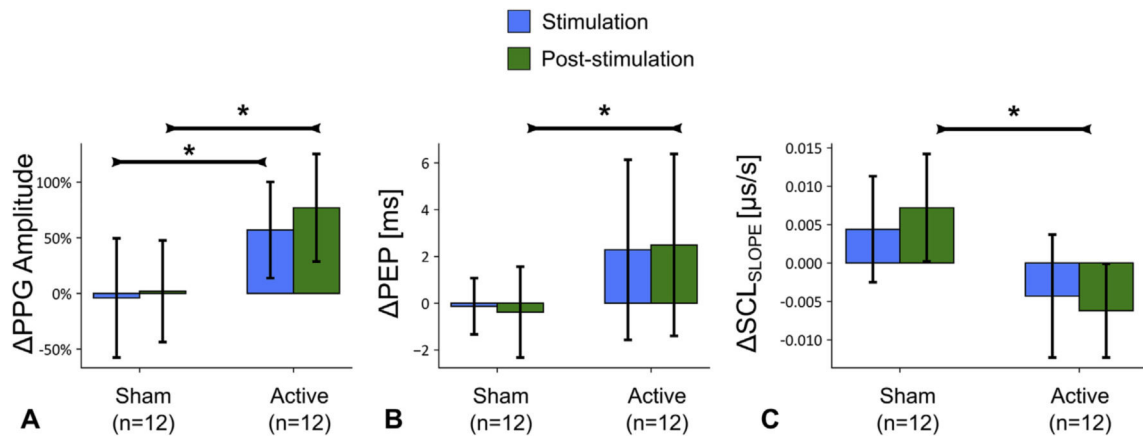
Fig. 3.
Primary outcomes from physiological signal analyses for stimulation without acute stress
from the second and third protocol days. Bars represent the unadjusted mean changes from
baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. **(A)**
Active tcVNS group experienced an increase in PPG amplitude during stimulation (p =
0.049) and post-stimulation (p = 0.021) compared to the sham group. **(B)** Active tcVNS
group experienced an increase in pre-ejection period during the post-stimulation interval (p
= 0.035) compared to the sham group. **(C)** Active tcVNS group experienced a decrease in
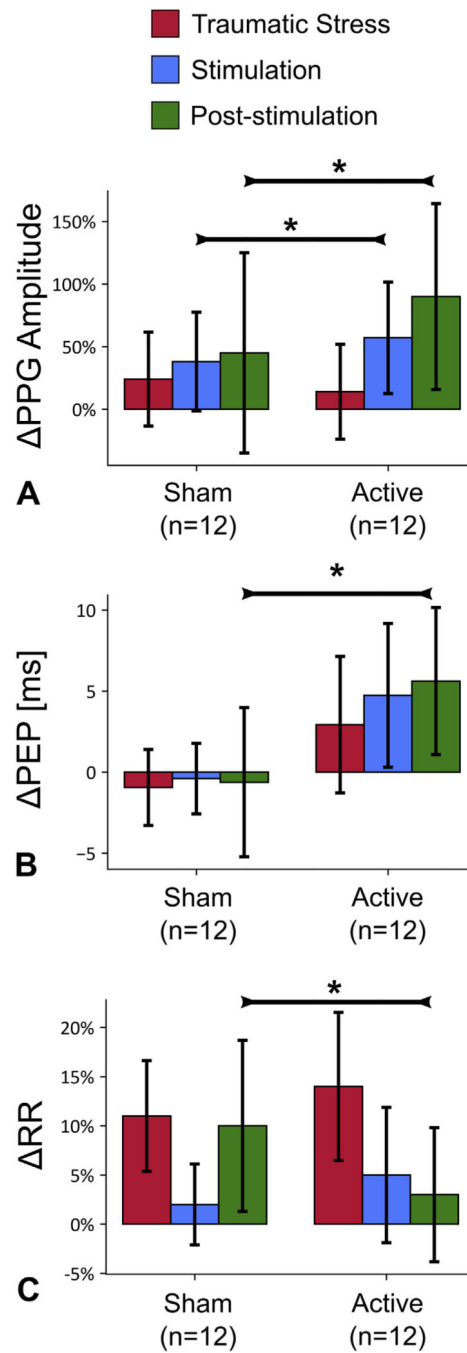SCL slope during the post-stimulation interval (p = 0.014) compared to the sham group.

Fig. 4.
Primary outcomes from physiological signal analyses for stimulation following traumatic stress. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. **(A)** The active tcVNS group experienced a greater increase compared to sham in PPG amplitude during stimulation ($p = 0.036$) and post-stimulation ($p = 0.044$). **(B)** The active tcVNS group experienced an increase in pre-ejection period during post-stimulation ($p = 0.003$) compared to sham. **(C)** Sham group experienced increase in respiratory rate (RR) during post-stimulation ($p = 0.002$).
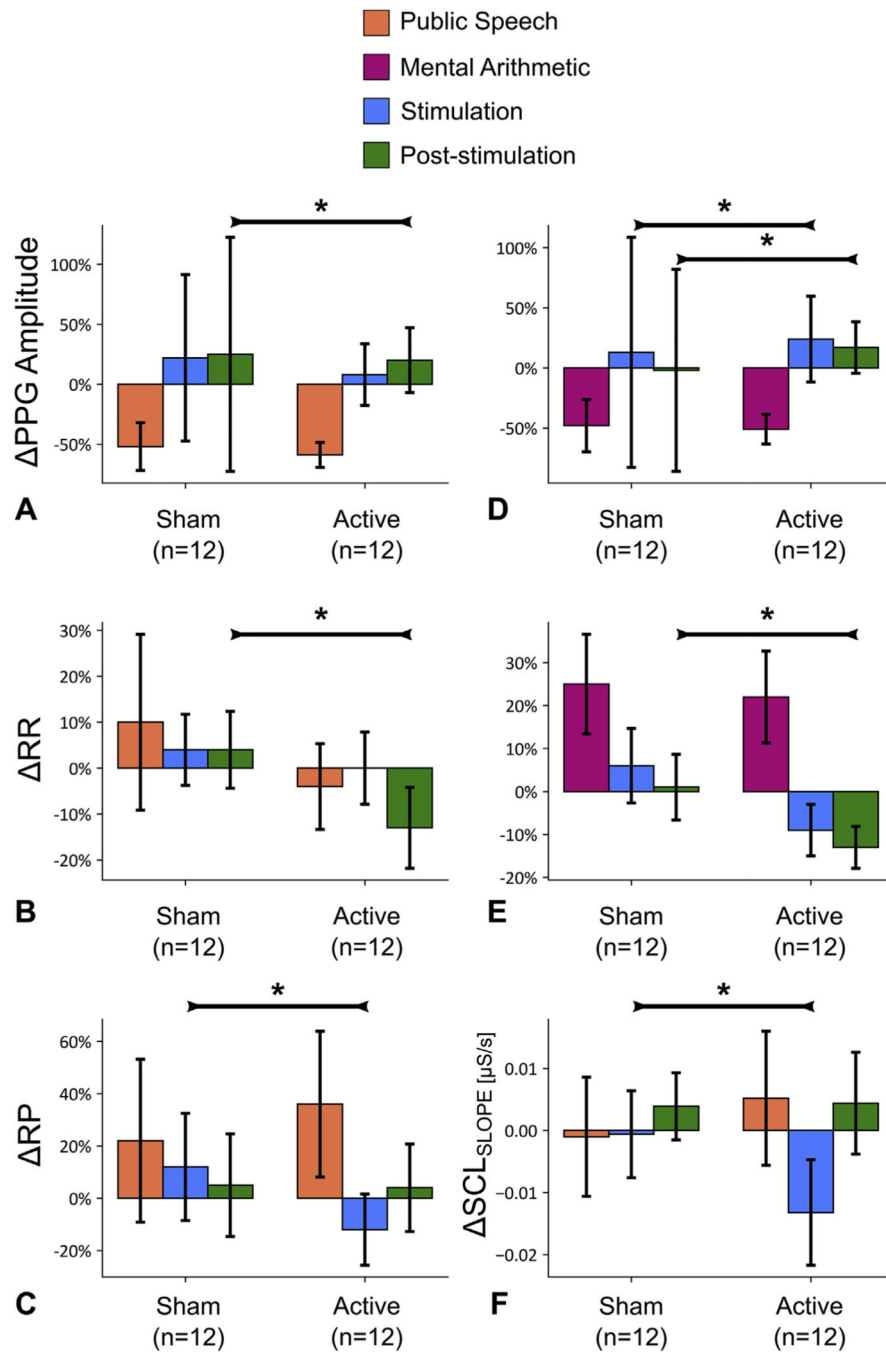
Fig. 5.
Primary outcomes from physiological signal analyses for stimulation following two types of
mental stress, public speech and mental arithmetic. Bars represent the unadjusted mean
changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p <
0.05$. **(A)** Increase in PPG amplitude for active group during post-stimulation ($p = 0.009$).
(B) Decrease in respiratory rate (RR) for active group during post-stimulation ($p = 0.017$).
(C) Decrease in respiration prominence (RP) for active group during stimulation ($p = 0.028$).
(D) Similar to **(A)**, active group shows a consistent recovery in PPG amplitude during

stimulation (p = 0.005) and post-stimulation (p = 0.001). **(E)** Decrease in RR during post-stimulation for active group (p = 0.007). **(F)** Decrease in SCL slope for speech task during stimulation for active group (p = 0.027).